VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

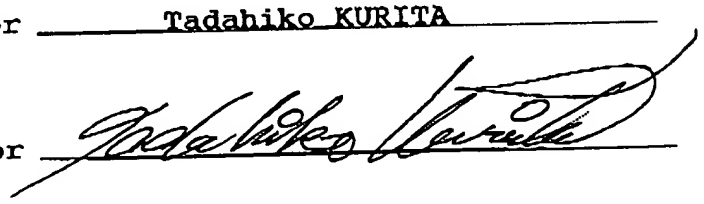
That I am knowledgeable in the English language and in the language in which the below identified application was filed, and that I believe the English translation of the Japanese Patent Application No. 272425/1996 is a true and complete translation of the above-identified Japanese Patent Application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

21st day of May, 2002

Full name of the translator Tadahiko KURITA

Signature of the translator 

Post Office Address c/o YUASA AND HARA, Section 206,
New Ohtemachi Bldg., 2-1, Ohtemachi
2-chome, Chiyoda-ku, Tokyo, JAPAN

PATENT OFFICE
JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: October 15, 1996

Application Number : Patent Application No. 272425/1996

Applicant(s): EBARA CORPORATION

Dated this 9th day of January, 1998

Commissioner, Hisamitsu ARAI (Seal)
Patent Office

ISSUANCE No. 09-3109322

[Name of Document] Patent Application

[Case No] P-23980

[Filing Date] October 15, 1996

[To] Commissioner, Patent Office

[Title of the Invention] BIOLOGICALLY ACTIVE POLYMER PRODUCTS

[Number of the Claims] 7

[Inventor]

[Address] c/o EBARA RESEARCH CORPORATION
2-1, Honfujisawa 4-chome, Fujisawa-shi
Kanagawa-ken

[Name] Yasuo FUKAGAWA

[Inventor]

[Address] c/o EBARA RESEARCH CORPORATION
2-1, Honfujisawa 4-chome, Fujisawa-shi
Kanagawa-ken

[Name] Akiko MIYA

[Applicant for Patent]

[Identification No.] 000000239

[Appellation] EBARA CORPORATION

[Representative] Shigeru MAEDA

[Proxy]

[Identification No.] 100073874

[Patent Attorney]

[Name] Taira HAGINO

[Telephone] 03-5561-3990

[Proxy]

[Identification No.] 100081075

[Patent Attorney]

[Name] Kiyotaka SASAKI

[Telephone] 03-5561-3990

[Proxy]

[Identification No.] 100066429

[Patent Attorney]

[Name] Toshio FUKAZAWA

[Telephone] 03-5561-3990

[Proxy]

[Identification No.] 100093573

[Patent Attorney]

[Name] Zenichi SOEDA

[Telephone] 03-5561-3990

[Fees]

[Page No. of Books] 008763

[Amount] ¥21,000.-

[List of the Documents]

[Item] Specification 1

[Item] Drawings 1

[Item] Abstract 1

[General Power of Attorney No.] 9005854

[Proof, requested or not] requested

[Name of the Document] Specification

[Title of the Invention]

BIOLOGICALLY ACTIVE POLYMER PRODUCTS

[Claims]

[Claim 1] A biologically active polymer product having:

a polymer substrate; and

a biologically active compound moiety having low molecular weight, the moiety being bonded to the polymer substrate and exerting selective biological activity,

wherein the biologically active compound moiety exerts the selective biological activity upon allowing the product to contact with a target substance while the active compound moiety is bonded to the polymer substrate.

[Claim 2] The biologically active polymer product according to claim 1, wherein the polymer substrate comprises an organic polymer or an inorganic polymer.

[Claim 3] The biologically active polymer product according to claim 2, wherein a graft chain is linked to the surface of the polymer substrate.

[Claim 4] The biologically active polymer product according to any one of claims 1-3, wherein the biologically active compound moiety is a chemotherapeutic.

[Claim 5] The biologically active polymer product according to claim 4, wherein the chemotherapeutic is an antibiotic.

[Claim 6] The biologically active polymer product according to claim 5, wherein the antibiotic is at least

one antibiotic selected from a group of beta lactam antibiotics.

[Claim 7] The biologically active polymer product of claim 6, wherein the antibiotic is at least one antibiotic selected from the group consisting of tetracycline antibiotics, chloramphenicol antibiotics, macrolide antibiotics and aminoglycoside antibiotics.

[Detailed Description of the Invention]

[0001]

[Field of Industrial Application]

This invention relates to a biologically active polymer product, and particularly, it relates to a polymer product wherein an immobilized, biologically active compound moiety exerts selective biological activity while being covalently bonded to a polymer substrate.

[0002]

[Prior Art]

Thus far, in research and development of drugs having biological activities, drugs having greater specificity against targets have been searched for better selective toxicity. On the other hand, with respect to known drugs, they are being used in certain controlled places and technology is being developed to reduce their influence on organisms other than those targeted. On this basis an effort is being made to suppress the adverse influence of residual drugs on the environment to the minimum. For example, a known scheme is that the combination of a harmful-insect attractant and an insecticide is used to

terminate only a harmful insect target by attracting it to the insecticide. However, with respect to microorganisms, technology has not yet been developed to use such drugs that attract only the target microorganisms and kill them alone. Therefore, there are no available methods for controlling the target microorganisms, other than to singly use drugs having selective toxicity against them.

[0003]

Under these circumstances, where raw materials for agriculture, forestry and fishery, and foods are manufactured in large quantities, chemotherapeutics that were originally developed for the emergency treatment of human or animal subjects are used as such, or used after their partial modifications; and organic, synthetic agrochemical with low selectivity of action (insecticides, bactericides, herbicides, oyster-attaching preventers, etc) are used. This not only gives drug-induced sufferings, which can not be ignored, to those other than the targets, but also induces the appearance of tolerant organisms due to the diffusion of the drugs within an environment. A serious danger is thus pointed out that the drugs, which were developed for the emergency treatment of humans, can no longer be employed in their intended therapeutic use. In other words, drug-resistance, especially resistance to a great variety of antibiotics (multiple drug resistance) prevails among the group of non-pathogenic microorganisms that constitutes an overwhelming majority in the ecological system of nature, and it is also amplifying propagation of

the multiple drug resistance among pathogenic bacteria. (M. Yoshikawa, Bull. of Jap. Soc. of Microbial Ecology. 10, No. 3, 141-148 (1995).)

[0004]

In medical treatment also, for example, anticancer drugs are expected to exert useful, selective toxicity effects only on cancer cells. However, when known anticancer drugs are actually administered to human subjects as such, they, as opposed to expectations, act not only on the cancer cells, but also on normal cells to bring undesirable adverse reactions on the patients. This, therefore, necessitates various considerations in their administration. Known as an example to be mentioned is a method of the drug use: in the clinical care of cancer by an anticancer drug, an anticancer drug the free diffusion of which is significantly restricted is placed on the affected part, and the drug is allowed to be released slowly and locally so that the adverse reactions of the drug effecting on normal cells can be alleviated. For example, an anticancer drug such as lomustine is sealed in capsules which comprise high-molecular weight polylactic acid that is hydrolyzed non-enzymically in vivo. Alternatively mitomycin C, an antitumor antibiotic, is processed such that it is allowed to be linked to a carboxyl side chain of dextran which is hydrolyzed enzymically in vivo (M. Hashida et al., Chem. Pharm. Bull. 2951 (1982)). These result in controlled-releasing medicinal preparations; and methods for their use in

therapy can be mentioned among others. Nevertheless, even in these cases, because the drugs exert anticancer activity by being liberated in vivo, their adverse reactions on normal cells may be alleviated, but can not be eliminated.

In the traditional concept, it has been fundamentally believed that drugs can not exert their intrinsic effects if they have been converted to the immobilized state, and that they can do so only in cases where they are used in their free state. In the case of medicinal preparations where low-molecular weight drugs are immobilized to polymer substances, it has been believed that the liberation of the drugs is an essential prerequisite when they are allowed to act on targets like the controlled-releasing preparations stated above.

[0005]

[Problems to be Solved by the Invention]

All drugs (which mean compounds, including from toxic substances to therapeutic agents, that have a wide spectrum of physiological activity) exist in a space or region as their free or dissolved state and move and diffuse freely within their environments. In pharmaceutical sciences, the fundamental mechanism of action that the drugs reach agents, which are the targets of their use, and exert their characteristic biological activities was therefore an essential prerequisite for use of the drugs. For this reason, when drugs are used, they not only act on the targets, but also have some influence (side effect) on all the agents coexisting within their environments more or

less. This was unavoidable in principle.

For instance, antimicrobial agents that are generally referred to as "disinfectants," "bactericides," "antimold agents," or the like are necessary commodities in daily life of the present. However, they are deficient in selectivity of biological action; and they are released within their environments because of the drugs' intrinsic solubility and free diffusibility to cause their serious accumulation within the environments, which has been bringing accumulative, adverse effects on humans, animals, and plants over a prolonged period of time.

[0006]

On one hand, particularly from the standpoint of the direct exclusion or alleviation of toxicity in the living things that are the subjects of treatment such as humans or cattle, a policy has been adopted in the research, the development, and the use of chemotherapeutics to date: the drugs are selected such that their biological activities are limited to their targets as much as possible and they only bring low adverse reactions at zero or tolerable levels on those other than the targets (high selectivity of action). Even in case, the drugs are inevitably diffused and remain within their environments as a result of their use, it can be avoided to irreversibly destruct the ecological system of the environment in which a wide diversity and variety of organisms exists as mingled and which functions normally while maintaining delicate balance, if the drugs have the high selectivity of drug action. For

example, the living bodies of humans and animals are where the respective ecological systems of microorganisms peculiar to them are functioning; once the balance of the ecological systems of the microorganisms is lost owing to imprudent drug use, harmful microorganisms that have not become dominant species before multiply, which may cause new serious disorders. An increase in the frequency of onset of opportunistic infections (endogenous infections) can be mentioned as an example of the consequence.

As has been stated above, all the drugs such as bactericides, insecticides, antimold agents, and agrochemical that have been used up till now and that have low selectivity of action, in addition to chemotherapeutics that have considerable selectivity of action diffuse into their environments regardless of their selectivity of action (high or low), since they are used in their free state; and they bring influence, which can not be ignored, on ordinary organisms or cells other than the target organisms, which turns to be the cause of destruction of normal balance of the ecological system.

[0007]

In these days when the environmental degradation or the adverse reactions of drugs have become a serious public concern, if a novel fundamental prerequisite in pharmaceutical sciences which is the drug use under conditions causing no free diffusion is hypothesized in addition to the conventional, fundamental prerequisite in pharmaceutical sciences which is the drug use under the

conditions of free diffusion, and concrete methods for utilization of the drugs are developed, then it can provide a new solution to the problems of environmental contamination and medical treatment: so contemplated the present inventors.

If another expression is employed from the standpoint of the utility of drugs, the emergency use of chemotherapeutics in their free state according to the traditional concept is unavoidable when the drug treatment is urgently needed to save the lives of specific individuals such as humans or animals that have been afflicted with acute infections by pathogenic bacteria, for example. However, where large amounts of drugs is allowed to diffuse into the natural environment for which the main purpose is a preventive effect by the indiscriminate killing of environmental microorganisms, it is desirable to cease the use that permits the drugs to diffuse freely.

[0008]

Further, if in the emergency drug treatment of humans or animals, drugs such as anticancer agents, insecticides, and bactericides that are restricted in their use, or can not be used because of severe adverse reactions through conventional systemic administration methods can be prevented from freely diffusing into their environments, according to this invention and can be used with drug-induced sufferings being suppressed at negligible levels, then novel methods for the utilization of medicines and agrochemical can be provided.

Thus, this invention aims at improving the environmental degradation and adverse reactions.

[0009]

[Means to Solve the Problems]

The inventors of this invention found the following facts and, on the basis of the facts, completed the present invention.

If a low molecular weight, biologically active compound is linked to a polymer substrate under the conditions described below, the active compound can exert its biological activity in the linked state and therefore the function of the active compound-substrate product can be evaluated. Those conditions are as follows:

① the polymer substrate per se is stable under the condition of actual use and is not decomposed;

② the active compound is linked to a substrate through a linking manner selected from some linking manners by which the compound can be linked to the substrate so that the activity of the active compound in the linked state can be exerted; and

③ environment is suitable for the active compound, which is linked to the substrate, to exert its activities. It is confirmed that the active compound linked to the polymer substrate is not diffused at al in environment during using it.

[0010]

The problems can be solved by a biologically active polymer product according to the present invention. That

is,

(1) A biologically active polymer product characterized in that:

a low molecular weight compound having selective biological activity is linked to a polymer substrate and that the low molecular weight compound linked to the polymer substrate exerts selective biological activity when the active compound contacts with a target substance.

(2) The biologically active polymer product of above (1) wherein the polymer substrate is an organic or inorganic polymeric substance.

(3) The biologically active polymer product of above (2) wherein the organic or inorganic polymeric substance is a grafted polymer.

(4) The biologically active polymer product of anyone of above (1)-(3) wherein the biologically active compound is a chemotherapeutic.

[0011]

(5) The biologically active polymer product of above (4) wherein the biologically active compound is an antibiotic.

(6) The biologically active polymer product of above (5) wherein the antibiotic is at least one antibiotic selected from the group of beta lactam natibiotics.

(7) The biologically active polymer product of above (5) wherein the antibiotic is at least one antibiotic selected from the group consisting of tetracycline antibiotics, chloramphenicol antibiotics, macrolide antibiotics and aminoglycoside antibiotics.

[0012]

One of the essentially important matters of the present invention resides in the basic conception. In the traditional concept, it has been fundamentally believed that drugs can not exert their intrinsic effects if they have been converted to the immobilized state, and that thy can do so only in cases where they are used in there free state in a region wherein they act on a target site and they freely move and diffuse to reach the site.

Accordingly, biological activities of a drug had been evaluated by measuring a titer through a dilution method or diffusion method under the presupposition that the drug acts in a free state or in a dissolved state.

In contrast, the inventors first found that a low-molecular weight drug which is linked to a polymer substrate and immobilized can be evaluate its biological activities and can exert the activities at a non-free state. In fact, no drug has been evaluated with respect to its at an immobilized state where the drug is linked to a polymer substrate.

[0013]

In the past, it was widely believed that when a drug was immobilized to a polymer through a chemical reaction, the selective biological activity peculiar to the immobilized drug could not be manifested unless the drug was liberated.

The present inventors discovered and confirming the following:

If a low-molecular weight drug is linked to a polymer substrate through a site of the drug where does not affect the selective biological activities of the drug, the drug can exert its activities while being linked to the substrate. Thus, a drug linked to a polymer substrate having selective biological activities peculiar to the drug can be obtained.

One example will be explained by way of a preparation obtained by immobilizing ampicillin, to a polymer, which serves as a polymer substrate. When the amino group of ampicillin was allowed to be linked to a carboxyl group of the substrate side through an amido linkage as is shown in FIG. 1(A) as attached, the antimicrobial activity peculiar to ampicillin was manifested; whereas, when the carboxyl group of ampicillin is allowed to be linked to an amino group of the substrate through an amido linkage as is shown in FIG. 1(B), the immobilized ampicillin displayed no antimicrobial activity at all.

Thus, the inventors found that a low-molecular weight drug can be linked to a polymer substrate at a moiety of the dug (or specific moiety) which does not affect its own activities. Of course, specific moieties are peculiar to types of drugs and therefore an example of ampicillin stated above should not be construed to limit the present invention. The number of specific moieties of drugs is not always one but may be two or more. The radicals to be useful to form linkage are not limited to a combination of a carboxyl and amino groups, but may other combination can

be used.

[0014]

In the past, a chemical reaction has been used to link a drug to a substrate. However, if a chemical reaction is simply applied to a drug and a substrate to form linkages, the drug will lose its activities as shown in FIG. 1(B) and therefore, it is impossible to obtain a drug linked to a polymer substrate which maintains its selective biological activities.

According to the present invention, a drug linked to a polymer substrate which maintains its biological activities can be obtained without causing deactivation.

[0015]

Here, the expression "exerts (the) selective biological activity" means that substantially no harm or tolerable adverse reactions are brought to a host environment (nature, a host animal or plant, cells, and organelle, etc.) and that the biological activity is exerted against a harmful, target agent the elimination or the control of which is wanted. Therefore, chemotherapeutics are mentioned as the biologically active compounds exerting their selective biological activity. The chemotherapeutics are drugs having high selective toxicity that causes only damage to harmful agents to be targeted without damaging hosts. The objects of chemotherapeutics are not limited to living things such as pathogenic microorganisms or harmful animals and plants, but extend to a considerably wide range, including cells

such as malignant tumors, and non-living matters such as enzymes, receptors, and hormones.

[0016]

To exert desired selective biological activity peculiar to a drug, the biologically active polymer product of this invention, preferably, not only satisfies the requisite conditions on the mode of linking between drug and substrate, but also has a microenvironment of action or a physical space suitable for the manifestation of effects by the drug immobilized to the substrate. Such physical space that is necessary for drug action can be created by the side chain on the polymer substrate which is generally referred to as a "spacer." The spacer which can be used in the invention preferably has a functional group capable of covalently and stably linking a drug molecule, as well as a chain having the length and properties necessary for manifestation of the selective biological activity peculiar to the drug, while the immobilized drug molecule is as it remains. When a spacer is to be provided, a biologically active compound may be linked to the spacer portion that has previously been linked to a polymer substrate; alternatively, a biologically active compound having previously linked to the spacer may be allowed to be linked to a polymer substrate at the tip of the spacer. However, the spacer is not limited to the graft chain being branched from a polymer substrate, which will be described later, nor is it limiting insofar as it provides the gap between the linked drug molecule and the substrate with an

appropriate distance and environmental nature.

[0017]

[Embodiments for Carrying out the Invention]

(1) Law-Molecular Weight Biologically Active Compounds

In carrying out this invention, it is, first, necessary to select a biologically active compound having desired biologically activity against a target. Second, basic information on the chemical structure-activity relationship of the selected biologically active compound is collected.

More specifically, once the target is first decided, a group of candidate drugs that is considered appropriate is selected, for example, from a known drug database, using their biological activity against the target as an index. Then, the most suitable compound can be determined from the group of candidate drugs according to conventional evaluation-testing methods; or unknown drugs having novel pharmacological activity against the target can also be screened.

Secondly, the structure-activity relationship of the selected biologically active compound is investigated; and such functional group or atom at a specified position (position which serves as the active site for immobilization) that the biologically activity peculiar to the drug does not abate even when other compounds are allowed to covalently be linked to the molecule of the selected drug is, preferably, selected.

[0018]

When no functional group available for linking to other compounds is found as the original chemical structure of the biologically active compound remains intact, a new functional group can also be introduced to the drug according to techniques in organic chemistry or in biochemistry.

As is clear from the results of synthesis of pharmacological compounds and their modification, and as is clear from the fact that immobilization of a low-molecular weight pharmacological compound linking to a polymer substrate was not studied, it is broadly known among researchers that easy modification and/or immobilization of drugs causes the deactivation of drugs.

[0019]

Among preferable drugs which can be utilized in this invention, those described below can be illustrated as the most important chemotherapeutics:
griseofluvin, vernamycin B, ostreogrycin G, isoniazid, pyridoxine, PAS, pimaricin, fungichromin, formycin, toyocamycin, chloramphenicol, tetracycline, streptomycin, erythromycin, ampicillin, norcardicin, SQ 83360, and OA-6129, etc.

[0020]

Among these chemotherapeutics, preferred are beta lactam antibiotics, tetracycline antibiotics, chloramphenicol antibiotics, macrolide antibiotics, and aminoglycoside antibiotics, etc. Ampicillin, cephalexin, cefotaxime and the like can be mentioned as examples of the

beta lactam antibiotics. In addition, minocyclin or the like can be mentioned as an example of the tetraccycline antibiotics, chloramphenicol or the like as an example of the chloramphenicol antibiotics, streptomycin or the like as an example of the aminoglycoside antibiotics, erythromycin, leucomycin, oleandomycin or the like as an example of the macrolide antibiotics.

[0021]

(2) Polymer Substrates (Matrices)

In this invention, the polymer substrate that is used to allow the linking of a biologically active compound exerting selective biological activity (which hereinbelow may be simply referred to as "substrate") is not particularly limited if it is a polymer that does not render the drug diffusive by decomposing or releasing it during the period when the biologically active compound is exerting its biological activity (i.e., during the effective period of product). Therefore, either an organic polymer or an inorganic polymer may be good for the polymer substrate of the invention. Although a variety of substrates can be used, the desirable characteristics with which the substrate should be provided can be, for example, referred to those described in Pure and Appl. Chem. Vol. 67, No. 4, pp. 597-600. Specifically, they are as follows:

i. Insolubility under the use conditions;

ii. Large capacity of immobilization; and

iii. Chemical inertness and strong mechanical strength.

[0022]

The substrate in this invention may be selected depending on the method for immobilizing the biologically active compound, as well as on the utility of the product.

First, it is necessary to select a substrate suited to the mode and conditions of immobilization reaction and to an environment for its use. For example, a physicochemically stable inorganic or organic polymer must be selected if severe conditions of use such as high temperature, high pressure, strong acidity, and strong alkalinity are anticipated; and a natural polymer may preferably be selected if the treatment of humans or animals is supposed.

Secondly, the selected substrate preferably has a functional group suited to immobilizing the selected biologically active compound stated above.

Furthermore, in order for the biological activity to be exerted while the biologically active compound is immobilized, the provision of a spacer is practically desirable that can create an adequate distance from the substrate and a microenvironment of action.

[0023]

(2-1) Substrates Comprising Organic Polymers

The organic polymers are preferably synthetic organic polymers, or may be resins. The substrate comprising the organic polymer may easily be processed into a membranous, reticulate, or spherical form.

(2-1-1) Substrates Comprising Organic, Synthetic Polymers

The organic synthetic polymers, which can be

mentioned, are, among others, as follows:
synthetic vinyl polymers, including polyolefins such as polyethylene and polypropylene, polyethers such as polyethylene oxide and polypropylene oxide, polyacrylic acids such as polyacrylonitrile, polyacrylates, and polymethacrylates especially, poly(hydroxyethyl)methacrylates, polyacrylamide, vinyl copolymers such as polystyrene, poly(vinyl acetate), poly(vinyl alcohol), ethylene vinyl acetate copolymer, poly(vinyl acetate styrene); and condensation polymers such as polyesters and nylons.

[0024]

(2-1-2) Substrates Comprising Other Organic Polymers

Mentioned as other organic polymers are natural organic polymers and modified natural organic polymers, which have been conventionally used to form the substrates for gel filtration, ion-exchange chromatography, affinity chromatography or the like. To specifically name these: cellulose, agarose, glucomannan, chitosan, pullulan, starch, dextran, etc. Conventionally, the substrates for use in affinity chromatography and the like have been processed as porous spheres of these natural organic polymers.

In contrast, mentioned as the materials that can be processed into fibrous or membranous forms are natural, organic polymers such as cellulose, which constitutes cotton fibers, flax fibers or the like, and linear proteins, which constitute wool fibers, silk fibers or the like.

[0025]

(2-2) Substrates Comprising Inorganic Polymers

Mentioned as an inorganic polymer is silicone resin (also referred to as "silicone"). To activate a polymer comprising linear silicone resin as its backbone, the following method is, for example, mentioned: a silicon compound, which constitutes the principal chain of the silicone resin, is made into hydrosilylated silicon; alternatively, silicone, which has hydroxylated silicon as the constituting unit at branching sites, is used, epichlorohydrin is allowed to act on this, and thus the hydrosilylated portion is activated through epoxidation to form an epoxy-activated silicone.

[0026]

(2-3) Preferred Processed Forms of Substrate

The synthetic or natural organic polymers, or the inorganic polymers can be shaped and processed into fibrous, membranous or spherical forms depending on the purposes of their use. In addition, those in the form of fiber can be processed into reticulate forms of various meshes for use. Furthermore, the substrate in the form of fiber, membrane, or sphere may be porous.

These substrates may be held by supports, aggregates or the like that comprises other metals or ceramics, or other materials.

The biologically active polymer product of this invention may directly link a biologically active compound through a suitable spacer by utilizing active sites of a polymer or other polymer that comprises the skeleton of the

fibrous form substrate, the membranous form substrate or the spherical form substrate; or it may link a low-molecular pharmacological compound by branching the polymer that comprises the skeleton of the substrate into graft chains and by utilizing the active sites (functional groups for linking) provided on those graft chains.

[0027]

(2-4) Graft Formation of Polymer Substrates

In immobilizing a biologically active compound to the polymer substrate, it is preferred that graft chains having suitable functional groups (e.g., carboxylic acid or amino group) be linked to the polymer substrate to effect graft polymerization.

The method of providing active sites for linking graft chains to the polymer substrate can utilize a method that is generally used, such as treatment by irradiation with ionized radiation or oxidation treatment with one or more kinds of oxidants. Especially preferred is a method of irradiation with ionized radiation using electron beam or cobalt 60 as a ray source. When the substrate is synthesized by vinyl polymerization, there may be a method in which a copolymerization monomer is mixed to cause copolymerization and to provide active sites comprising vinyl groups at the side chains of the substrate polymer. In addition, a liquid-phase graft polymerization method, a gas-phase graft polymerization method or the like can be employed as the graft polymerization method; and generally preferred is the gas-phase graft polymerization method that

makes the monomer into its gas-phase state and contacts it with the substrate.

[0028]

In the graft formation, graft chains formed therefrom can be provided with the role as the spacer. Since a biologically active compound is linked to the terminus of, or along the graft chain, the biologically active compound molecule that is linked is to be disposed at a position away from the substrate, and the biologically active compound can have an interaction space and a microenvironment that are requisite for manifestation of the biological activity peculiar to the drug. Particularly, if the graft chain is sufficiently elongated so as to be able to move according as the conditions such as the flow of fluid or the concentration gradients of substance, the probability of contact between the immobilized drug molecule and the target can be enhanced. Further, the biologically active compound links not only to the terminus of a graft chain, but also to a pendant along the graft chain; therefore, a longer graft chain may normally be linked to many biologically active compounds and through the optimization of arrangement of the graft chains, as well as the number of pendants, the utilization efficiency of the drug can be enhanced.

[0029]

The functional group that is provided along or at the terminus of the graft chain arranged on the polymer substrate to immobilize a biologically active compound is

optimized depending on the type and mode of the functional group on the biologically active compound molecule. For example, if a carboxyl group (or an amino group) is provided at the graft side chain, a stable peptide linkage can be constructed by the carbodiimide method using an amino group (or a carboxyl group) of the biologically active compound.

[0030]

(3) Types and Modes of Linkages for Use in the Immobilization of Biologically Active Compounds

As already stated, the linkages that can be used for the immobilization of biologically active compounds in this invention must be strong covalent bonds so that liberation of the biologically active compounds might not occur in their use environments. Although the definition of conventional immobilization embraces linking techniques such as embedding, ionic linking, milling, and nonspecific adsorption, naturally they can not be used in the invention supposing that liberation of the biologically active compounds into the environment would not occur. Therefore, the linking between the biologically active compound and the polymer substrate employ a covalent bond in the invention.

The "sustained release" covalent linkage of a pharmacological compound to a polymer substrate which has been broadly used for that purpose is not useful to the present invention because such a linkage is intentionally used to slowly release the active compound which acts in a

free state. Thus, the covalent bond to be used should be one that the release of the active compound is not released. It is necessary to choose an appropriate type of covalent bonds by taking into consideration of specific type of functional groups of the active compound and a polymer substrate and of the spacer, type and mode of the intended bond, or the like.

[0031]

A spacer that is necessary for linking the biologically active compound to the polymer substrate has been provided on the substrate side, and then, the biologically active compound can be immobilized to the terminus of the spacer. Alternatively, a spacer has been attached to a selected active site of the biologically active compound, and then, the low-molecular pharmacological compound having the spacer can be immobilized to the "substrate having an active site" through the spacer.

The main portion of a suitable spacer that can be utilized in this invention may be any chain form insofar as it can link "the active site provided on the substrate" or "the active site provided at the graft chain branched from the substrate" with "the functional group of the drug," and in addition, can effectively be used to give a microenvironment suited to the manifestation of action of the drug. Specifically, a straight methylene ($-CH_2-$) chain, a straight oxyethylene ($-O-CH_2-CH_2-$) chain, or the like can advantageously be used, but it needs to be a linking group

that would not cleave even under severe conditions such as high temperature, acidity, alkalinity, or the like.

[0032]

The functional groups that can be used at "the active site provided on the substrate," or at "the active site provided at the graft chain branched from the substrate," or at "both termini of the spacer (may not be the same groups)," or at "the specified position of the biologically active compound for linking" and that can be mentioned are, for example, as follows:
a hydroxy group, an amino group, an epoxy group, a glycyl group, an isocyanate group, an aldehyde group, a carboxyl group, a vinyl group or an allyl group.

In addition, as the active site in an inorganic polymer substrate, a hydrosilyl group in silicone resin can be mentioned.

[0033]

The active site provided on the substrate, the active site provided on the graft chain branched from the substrate, both termini of the spacer, or the functional group in a low-molecular pharmacological compound is an amino group and the low-molecular pharmacological compound having a carboxyl group as the activating group is to be immobilized; in such a case immobilization can be conducted using a carbodiimide condensation agent such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

Further, the active site provided on the substrate, the active site provided on the graft chain branched from

the substrate, both termini of the spacer, or the functional group in a low-molecular pharmacological compound is a hydroxyl group and the low-molecular pharmacological compound having an amino group as the activating group is to be immobilized; in such a case the terminal hydroxyl group is converted to a terminal aldehyde group by periodic acid oxidation, a Schiff base bond is then formed by the action of the low-molecular pharmacological compound having a terminal amino group, and the Schiff base bond is converted to a chemically stable carbon-nitrogen linkage ($-CH_2-NH-$) by being reduced, which can thus effect linking.

[0034]

Furthermore, in this invention there are some linkages that may not be used because of steric hindrance or unsuitability of the microenvironment where the drug manifests its biological activity in the product, depending on the kind and the linking mode of the low-molecular pharmacological compound. Therefore, it is necessary to determine the presence or absence of manifestation of any biological activity with respect to individual pharmacological compounds and substrates.

According to the present invention, a low-molecular weight pharmacological compound should be linked to a substrate at a site where does not affect its pharmacological activities when the compound is immobilized. In the past, it has not been fully studied about specific site of a low-molecular weight pharmaceutical compound

which derives its pharmacological activity. Accordingly, if the pharmacological compound is intended to be used as being carried on a substrate, there has been no conception as to which site of the compound should be used or how the linkage should be formed. Thus, no desirable method for formation of linkage of the compound to a substrate has not been proposed.

[0035]

In this invention, where a penicillin or cephalosphorin derivative having an amino group at its side chain and a carboxyl group on its mother neucleus such as ampicillin, cephalexin, or cefotaxime is allowed to be linked to the substrate, it has been ascertained that the drug exert its efficacy when the amino groups at the side chain is covalently bonded to the carboxyl group of the substrate while the carboxyl group of the penicillin or cephalosporin skeleton is maintained as such.

In FIG. 1(A), the amino group at the side chain of ampicillin is linked to the carboxyl group of the substrate by a carbodiimide method. In this instance, the linked ampicillin exerts its efficacy because the carboxyl group on its mother neucleus is maintained as such. In contrast, the carboxyl group on the mother neucleus of ampicillin is linked to the amino group of the substrate by the carbodiimide method in FIG. 1(B). In this instance, the immobilized ampicillin does not exert any efficacy because the carboxyl group is chemically denatured.

[0036]

The inventors found that such a low-molecular weight pharmacological compound can be firmly linked to a substrate at a site which does not affect its activity and generally employed the technique in the present invention. This technique makes the present invention novel. On the other hand, according to a conventional method, a low-molecular weight pharmacological compound is merely linked to a substrate by a chemical reaction. The site of the compound to be linked and the type of reaction for linking are not considered. Such a conventional method is clearly different from the present invention.

In the past, some products mobilizing a biological active compound were proposed. However, when producing such products, the linking site and the linking reaction were not considered and therefore, the proposed products lost the pharmacological activity of the low-molecular pharmacological compound because the site of the compound which affects the pharmacological activity is reacted. Alternatively, the pharmacological activities of the proposed products were blocked due to steric hindrance derived from the structure of the product. If the proposed product contains some linkages which are suitable to maintain the activity of the pharmaceutical compound, the product also contains undesired linkages and therefore the activity of the product is reduced as a whole.

For the reasons stated above, no desirable polymer product having a high pharmacological activity has been provided. According to the present invention, the above-

mentioned problems has been solved.

[0037]

A preferred embodiment of linking the biologically active compound to the substrate in this invention is as follows: in carrying out graft polymerization, the graft polymerization is conducted on the substrate provided with an active site to allow a graft chain to link to the substrate using a monomer, according to the method that will be explained below.

In conventional graft polymerization methods, the liquid-phase graft polymerization method that allows direct contact between substrate and monomer was popular. However, since large amounts of monomers and washing chemicals are needed, there is a disadvantage that the running cost becomes high. Especially, in the case of a porous substrate larger amounts are needed and besides, the time required for cleaning is lengthy. In contrast, the gas-phase graft polymerization method, by which a monomer is converted to its gaseous state and then it is allowed to contact with the substrate, needs only a very small amount of monomer and no washing; and the method is advantageous from the standpoint of time and cost, although the airtightness of a polymerization device requires attention. Further, the problem of ununiformity in polymerization that will become a concern in the gas-phase graft polymerization has disappeared due to an improvement on the polymerization device.

[0038]

In this invention, the polymer substrate that will form grafts may be either an organic polymer or an inorganic polymer. Organic polymers as stated above, include polyolefines; polyethers; polyacrylates; polystyrenes; synthetic vinyl polymers such as poly(vinyl acetate), poly(vinyl alcohol) and vinyl copolymers; polyesters; polyamides such as nylons; polyurethanes and the like. Organic natural polymers as state above include cellulose such as cotton fibers, flax fibers and the like; linear proteins such as wool fibers, silk fibers and the like. Modified natural organic polymers include acetylcellulose, acetylbutylcellulose, acetylphthalyl cellulose and the like. Inorganic polymer compounds include polymers having linear silicon resin as backbone.

[0039]

The functional groups as already described in the section of "Types and Modes of Linkages for Use in the Immobilization of Biologically Active compounds," among others, may be mentioned as the functional group for immobilizing a biologically active compound that is provided in or at the terminus of the graft chain provided on a polymer substrate. For example, when a carboxyl group (or an amino group) has been provided at the grafted side chain, this terminal group and the amino group (or the carboxyl group) of the biologically active compound can be linked, according to the carbodiimide method.

The concrete methods for providing an active site such as a carboxyl group or an amino group in or at the

terminus of the graft chain provided on the polymer substrate, which can be mentioned, are, for example, as follows:

(i) a method for graft polymerization using acrylic acid or methacrylic acid as a graft monomer or a graft copolymerization monomer (the functional group being a carboxyl group);

(ii) a method for graft polymerization using amino styrene as a graft monomer or a graft copolymerization monomer (the functional group being an amino group); and

(iii) a method for graft-polymerizing vinyl acetate with its use as a graft monomer or a graft copolymerization monomer, thereafter utilizing the hydroxyl group of vinyl alcohol formed as the result of the saponification of a vinyl acetate unit and substituting it with a spacer that has an epoxy group and an amino group at its termini (the functional group being an amino group).

Nevertheless, the method for providing an active site in the graft chain or at its terminus is not limited to those mentioned: they are adequately optimized depending on the chemical characteristics peculiar to selected biologically active compounds.

[0040]

Further, in this invention the immobilization of a biologically active compound has a purpose of preventing the biologically active compound from freely moving and diffusing within its environment. Therefore, when the biologically active compound is immobilized to the

spherical substrate described above, it is preferred that the immobilization be conducted so that the spherical substrate may not disperse into the treatment space (e.g., a filtration bed provided on the passageway for filtering the air containing treatment objects) of objects to be treated (bacteria, molds, etc).

[0041]

(4) Assays for Biological Activity

Conventionally, the following quantitative evaluation method has been adopted in the case of evaluation of the biological activities of drugs: assuming that a drug is dissolved in solution, the magnitude of its efficacy against a target (a test agent) is determined by the dilution method or the diffusion method, and the presence or absence of efficacy of the drug is evaluated based on its potency. However, since the biologically active compounds can not freely disperse with respect to the products of this invention, the test evaluation system prescribed by the Drugs, Cosmetics and Medical Instruments Act is not applicable.

In this invention, therefore, test conditions where a target is forcedly allowed to contact a test drug-immobilized polymer substrate are set, and the magnitude of its efficacy is quantitatively evaluated. Namely, the present inventors tested the functions of products according to a rapid evaluation method for antibiotic activities which will be explained below. First, to 5 ml of liquid medium are added one or two drops of a

preliminary culture solution of a test microorganism that has been shake-cultured from the middle period of its logarithmic growth phase up till a late period thereof, and a provided polymer product (section of 1.0 x 1.0 cm in the case of a product in the form of membrane). It is shake-cultured at a temperature suited to the growth of the test microorganism. For example, when Bacillus subtilis ATCC6633, Staphylococcus aureus FDA 209P, Escherichia coli NIHJ or the like is to be used as the test microorganism, a Nutrient Broth medium can be used as medium; and the culturing temperature of 30°C and the overnight culturing time prior to shaking can be employed.

[0042]

A polymer substrate (1.0 x 1.0 cm) that supports a biologically active compound to be a test subject is placed in a culture medium, 5 ml; one or two drops of an overnight-cultured solution containing a test microorganism is inoculated in it; absorbance is measured at 600 nm using a spectrophotometer; and the growth curve of the test microorganism is determined over 6 - 8 h. At the same time, carrying out similar manipulations without addition of the provided polymer product, the normal growth curve of the test microorganism is determined, which serves as an ineffective control.

Usually, the test microorganism culture solution reaches the logarithmic growth phase or the stationary phase after culturing for 6 - 8 h, which is the measuring time. At this point, if similar manipulations are carried

out in parallel on a polymer substrate linking to no drug, the activity of the drug linked to the polymer substrate can be evaluated more accurately.

In the rapid evaluation method for antibiotic activities, when the provided polymer product is in the form of particles, about 0.25 ml of the provided polymer product is used in place of a section of 1.0 x 1.0 cm^2 and similar manipulations are carried out. In addition, even when the provided polymer product is in the form other than membrane and particles, it can be evaluated according to a method similar to those for polymer products in the form of membrane or particles. In this instance, if the polymer product is dispersed within a medium and the growth of a test microorganism can not be monitored by the absorbance at 600 nm, said culture solution is sampled at suitable intervals to be observed under an optical microscope and the activity can be evaluated by comparing it with a test microorganism culture solution which is the control.

[0043]

The test microorganisms for use in this invention can employ microorganism cell strains that are used in ordinary evaluation of antibiotic activities, as well as standard cell strains preserved in various cell-preserving institutions and microorganisms isolated from nature. For example, the following can be mentioned: Staphylococcus aureus FDA209P; Staphylococcus aureus Smith; Staphylococcus aureus K2; Bacillus subtilis ATCC6623; Bacillus anthracis; Mycobacterium 607; Mycobacterium

ATCC15483; Mycrococcus luteus ATCC9341; Proteus vulgaris OX-19; Klebsiella pneumoniae PCI 602; Escherichia coli NIHJ; Escherichia coli JM109; Escherichia coli MV1190; Pseudomonas aeruginosa; Salmonella enteritidis; Candida albicans 3143; and Trichophyton mentagrophytes.

Further, in the rapid evaluation method for antibiotic activities, it is preferred that with respect to selected test microorganisms, their media, culturing temperatures, and culturing times prior to shaking, which are optimum for their growth, be set.

[0044]

Under the present circumstances, it is impossible to quantitatively evaluate the amounts of drugs immobilized to substrates, either chemically or biologically, with the accuracy that is comparable to test methods based on the diffusion method applicable to free drugs. Furthermore, as for the substrates to which drugs are immobilized, there exist no established theories that are scientifically accepted as far as methods for the quantitative evaluation of their activity as antibiotics are concerned.

In the test methods based on conventional diffusion methods applicable to free drugs, the prerequisite is that media, glass apparatuses and the like are sterilized, besides, the drugs themselves that are the subjects of evaluation are manufactured and stored aseptically. It is, therefore, possible to evaluate the effects of the drugs on the growth of selected test microorganisms accurately. In contrast, among polymer substrates that are the subject of

this invention, there are some of such nature that they can not be sterilized in an autoclave at 121°C. The rapid evaluation method for antibiotic activities of the invention, therefore, aims at the evaluation under the conditions where even if the method is subject to influence by microorganisms other than the test microorganism present in an environment such as air, the influence can be eliminated or ignored.

[0045]

The rapid evaluation method for antibiotic activities is an evaluation method similar to the shake flask method: it is adopted by the Fabric Product Hygienic Processing Council when the Council issues certification marks (SEK marks) to antimicrobial deodorizing processed fabric products, and mainly, it is an evaluation method for the effectiveness of products that are treated with processing chemicals of the non-elution type. (Techniques in the Use of Antimicrobial and Anti-Mold Agents, and the Testing and Evaluation of Antimicrobial Potency 244: the Technical Information Association Ed.; the Technical Information Association; 1996.) According to the shake flask method, a provided fabric product that is cut in about 1.0x1.0 cm and a provided microorganism culture solution are added to a flask to which phosphate buffer solution has been added and which is then sterilized in an autoclave at 121°C. After the body cells are allowed to contact the provided fabric product by shaking for 1 h, the number of viable cells is counted by the mixed dilution argar plain plate method, and

the viable cell number is to be compared to the control that has been treated similarly without addition of the provided fabric product.

[0046]

In the test method based on conventional diffusion methods applicable to free drugs, the time of contact between the test microorganism and the drug is maintained for 24 h or longer, and the effect on the microorganism is evaluated. In contrast, the condition that is common to the rapid evaluation method for antibiotic activities of this invention and the shake flask method is that the time of contact between the test microorganism and the drug is set to be brief. The reason is the following: Even where microorganisms other than the test microorganism are present in the culture solution at the start of testing, if their presence is very small when compared to the amount of addition of the test microorganism, it is thought that the influence of the microorganisms other than the test microorganism can be eliminated or ignored under this condition as long as the evaluation is conducted within the culturing time of 6 - 8 h during which an ordinary test microorganism reaches the latter period of its logarithmic growth phase or its stationary phase.

Additionally, in the rapid evaluation method for antibiotic activities, by using a spectrophotometer while comparing with the control that has no efficacy at all times, the absorbance of the test microorganism culture solution at 600 nm-wavelength is measured in a time-

dependent manner and the growth process of the test microorganism is monitored. Thus, the influence of microorganisms other than the test microorganism can be eliminated or ignored, and it can be said that the method is a reasonable evaluation method under the present circumstances.

[0047]

(5) Examples of Utilization of Biologically Active Polymer Products of the Invention

In this invention, a biologically active compound having selective biological activity is covalently bonded to a polymer substrate so as not to be liberated, thus making the biologically active polymer product. Accordingly, the characteristics that the biologically active compound linked to the polymer product is not liberated from the polymer substrate mean the following:
(i) No liberated biologically active compound remains in an environment that has been treated with the biologically active polymer product of the invention; and
(ii) It is required that the biologically active polymer product be contacted directly with a target.

[0048]

According to an example of utilization, when fluids such as blood, body fluid, water, drinks, air, foods, and feeds are forcedly conveyed and briefly contacted with the biologically active polymer products of this invention, targets can be removed from the fluids without the fear that liberated drugs might remain in the fluids after

treatment and might bring undesirable influence. For such utility, products of the invention such as those in the form of membrane, a net, or a spherical, filter medium may be used advantageously. In this case the targets that can be mentioned are, for example, a wide range of agents including living things such as viruses, microorganisms, pollen, eggs, insects, and small animals, besides non-living matters such as enzymes, hormones, and toxic substances.

[0049]

Another example of utilization of the biologically active polymer products of this invention is use forms that suppose the use for considerably long hours. For example, the polymer products of the invention that have bees processed into sheet forms, paint forms, or fibrous forms may be utilized as clothes, curtains, sheets, paint products, external films, sanitary goods, etc.

Thus, a variety of utilization is possible for the polymer products of this invention to which biologically active compounds having selective biological activities are linked, and the examples of their utilization are not to be limited to those illustrated herein.

The concrete utility of this invention is not limited to the fields of medical treatment or the manufacture in agriculture and fishery, and it extends more widely to preservation of the environment concerning, among others, daily life, breeding, and cultivation or water environments (including the preservation of water quality, the

prevention of abnormal growth of microorganisms, and the like).

[0050]

[Examples]

The examples of the polymer products of this invention which are produced by binding a low molecular weight compound to a polymer substrate will be illustrated below. Nevertheless, the invention is not to be limited by the examples that follow.

[0051]

Example 1 Grafted fibers with carboxyl groups Having Immobilized Ampicillin

A nonwoven fabric having a METSUKE of 50 g/m^2 and a thickness of 0.4 mm, which was made of polypropylene (available from Mitsui Petrochemical Ind. Co.; the trade name: Syntex PS-110), was irradiated 200 kGy with electron beam (at 2 Mev and 1 mA) under a nitrogen atmosphere. Then, these fibers were immersed in a monomer solution of acrylic acid/methanol (1/9) and were allowed to react at 40°C for 4 h. Consequently, fibers having an acrylic acid graft rate of 78.5% were produced, which are hereinafter referred to as the "grafted fibers with carboxyl groups." The ion-exchange capacity of the grafted fibers with carboxyl groups was 6 meq/g. Here, the graft rate was calculated according to the following formula:

graft rate={(weight of substrate after graft formation-weight of substrate before graft formation)/ weight of

substrate before graft formation}x100(%)

[0052]

The sodium salt of ampicillin (molecular weight of 349.42), 51.3 mg (equivalent to 48.2 mg as converted based on ampicillin free base), 40 pieces of grafted fibers with carboxyl groups that were cut into squares in 1.0 x 1.0 cm (total weight of 513 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (water-soluble carbodiimide), 252. 4 mg, were added to 50 ml of a M/100 potassium phosphate buffer (pH 4.4) in a 250-ml Erlenmeyer flask. Shaking was conducted at 5°C for 20 h (revolution radius: 12 cm, the number of revolution: 80 times/min), and ampicillin was immobilized on the grafted fibers with carboxyl groups.

As a control, a linking reaction containing no water-soluble carbodiimide (the grafted fibers with carboxyl groups, 496 mg, and ampicillin hydrochloride, 51.3 mg, were added to 50 ml of a M/100 potassium phosphate buffer (pH 4.4)) was carried out under the same conditions.

After the reaction was over, fibers were taken out and washed with 100 ml of purified water twice. Further, they were washed under shaking in 100 ml of purified water at room temperature for 3 h. To prevent contamination by various bacteria such as mold, an ampicillin-immobilized sample and a control sample that had been already washed were immersed in pure methanol immediately after washing and stored in a refrigerator.

[0053]

Elemental analysis values of sulfur (S) and nitrogen (N) for these ampicillin-immobilized grafted fibers with carboxyl groups and the control grafted fibers with carboxyl groups are as follows:

ampicillin-immobilized sample: S, 0.131%; N, 1.19%
control sample of grafted fibers with carboxyl groups : S, 0.00007%; N, 0.02%

Since S is derived from ampicillin, on the basis of the S elemental analysis values it is calculated that in this immobilization experiment a total amount of 7.41 mg of ampicillin was immobilized to 513 mg of the grafted fibers with carboxyl groups. Thus, it has been proved that 185 μg per piece of ampicillin is immobilized in a piece of the ampicillin-immobilized grafted fibers with carboxyl groups. Although the elemental analysis values for N are markedly high when compared to those for S, it has been deemed from previous findings that carboxyl groups which are maintained in the carbodiimde-activated state without being utilized in the immobilization of ampicillin coexist in large amounts.

[0054]

FIG. 1(A) shows the linking position and the mode of linking between ampicillin and grafted fibers with carboxyl groups. On the other hand, the one (FIG. 1(B)) in which the carboxyl group of ampicillin was linked to the amino

group of grafted fibers with amino groups could not exert the biological activity that was characteristic to ampicillin.

FIG. 2 shows the results from a test of antimicrobial activity of these ampicillin-immobilized sample and control sample against Staphylococcus aureus FDA 209P, as assayed by the rapid evaluation method for antibiotic activities. Further, under similar testing and evaluation conditions, this ampicillin-immobilized grafted fibers with carboxyl groups displayed antimicrobial activity against Bacillus subtilis ATCC6623, Bacillus anthracis, Staphylococcus aureus K2, Staphylococcus aureus Smith, etc. However, even this ampicillin-immobilized sample was ineffective against Escherichia coli NIHJ, Proteus vulgaris OX-19, Klebsiella pneumoniae PCI602, Candida albicans 3143, Aspergillus niger, Micrococcus luteus ATCC9341, etc. Namely, it can be said that the ampicillin-immobilized grafted fibers with carboxyl groups according to this invention exert the selective biological activity that is peculiar to ampicillin.

[0055]

An ampicillin antimicrobial spectrum which was measured by a conventional method for evaluating activities of antibiotics (Excerpt of Y. Ueda, K. Shimizu: "β-lactam antibiotics" pp. 164, 1987) is shown in Table 1. The ampicillin-immobilized grafted fibers of this invention had the same spectrum which is exerted by a free state ampicillin.

[0056]

Table 1

Antimicrobial Spectrum of Ampicillin

	final inhibition concentration (μg/ml)
Staphylococcus aureus FDA209P	0.015
Bacillus anthracis	0.031
Klebsiella pneumoniae	5.0

[0057]

Even when this ampicillin-immobilized grafted fibers with carboxyl groups was thoroughly washed with high purity deionized water until TOC (total concentration of organisms) of the washed solution reached about 20 μg/l which was equal to the blank value, it maintained antimicrobial activity at the same level as before thorough washing. It continued to maintain antimicrobial activity against Staphylococcus aureus FDA209P even after it was subject to heat treatment at 0°C for 10 min.

FIG. 3 results from an investigation of the correlation between the surface area of a sample immobilized product of ampicillin-immobilized grafted fibers with carboxyl groups and its antimicrobial activity. As is apparent from FIG. 3, the surface area of the sample of ampicillin-immobilized grafted fibers with carboxyl groups (i.e., the absolute amount of ampicillin on a piece of test sheet) has a positive correlation with the

antimicrobial activity.

Treatment with 1 M glycine, pH 8.5, and ethanol amine was conducted on these samples at room temperature for 3 h according to a standard method and unreacted carboxyl groups in the activated state were inactivated, but their antimicrobial activity did not change before or after the treatment.

[0058]

Example 2 Grafted fibers with carboxyl groups Having Immobilized Cephalexin or Cefotaxime

Substituting the sodium salt of ampicillin in the reaction mixture composition according to Example 1 with an equivalent amount of cephalexin or cefotaxime, it was immobilized to the grafted fibers with carboxyl groups under the same conditions. When the antimicrobial activity test was conducted using the rapid evaluation method for antibiotic activities, the cephalexin-immobilized grafted fibers with carboxyl groups as well as the cefotaxime-immobilized grafted fibers with carboxyl groups displayed distinct antimicrobial activity against Staphylococcus aureus FDA209P.

[0059]

Example 3 Ampicillin-Immobilized Agarose Beads of the Carboxylic Acid Type

A modified natural polymer substrate in the form of beads that used agarose as a base material and that had carboxyl groups as the functional group (the commercial name: ECH-Sepharose 4B), 0.5 ml, was suspended in purified

water at room temperature and washed. Then, it was suspended in a M/100 potassium phosphate buffer (pH 4.5), 5 ml. To this were added the sodium salt of ampicillin, 5 mg, and water-soluble carbodiimide hydrochloride, 25 mg, and it was gently stirred at 5°C for 6 h. After the immobilization reaction was over, the ampicillin-immobilized ECH-Sepharose 4B, 0.1 ml, that had been sufficiently washed with purified water was used and its antimicrobial activity against Staphylococcus aureus FDA209P was investigated according to the rapid evaluation method for antibiotic activities, when distinct antimicrobial activity was confirmed.

[0060]

Example 4 Ampicillin-Immobilized Synthetic Beads of the Carboxylic Acid Type

A porous synthetic resin of the carboxylic acid type in the form of beads (the commercial name: Daiaion WK10) was used as the polymer substrate. After this polymer substrate, 0.5 ml, was suspended in purified water at room temperature and washed, it was suspended in a M/100 potassium phosphate buffer (pH 4.5), 5 ml. To this were added the sodium salt of ampicillin, 5 mg, and water-soluble carbodiimide hydrochloride, 25 mg, and it was stirred at 5°C for 24 h. After the immobilization reaction was over, the ampicillin-immobilized Daiaion WK10 (0.1 ml) that had been sufficiently washed with purified water was used and its antimicrobial activity against Staphylococcus aureus FDA209P was investigated according to the rapid

evaluation method for antibiotic activities, which confirmed the manifestation of the antimicrobial activity.

[0061]

Example 5 Oxirane·Acrylic Acid Beads Having Immobilized Ampicillin

A polymer substrate that had a principal structure comprising methacryl amide and contained epoxy groups at the terminus of its side chains, and that had bead forms (the commercial name: Oypergid), 0.5 g, was suspended in purified water at room temperature and washed. Then, it was suspended in a M/100 sodium potassium phosphate buffer (pH 8.5), 30 ml. To this were added the sodium salt of ampicillin, 100 mg, and it was stirred at 25°C for 24 h.

After the reaction was over, unreacted epoxy groups were inactivated with 8 g glycin/100 ml 0.5 M phosphate buffer (pH 8) according to the instructions by the manufacturer of bead products. Ampicillin-immobilized Oypergid, 0.05 g, was taken, and after washing with purified water, its antimicrobial activity against Staphylococcus aureus FDA209P was investigated according to the rapid evaluation method for antibiotic activities, which confirmed the manifestation of the antimicrobial activity.

[0062]

Example 6 Cellulose Beads of the Carboxylic Acid Type Having Immobilized Ampicillin or Cephalexin

A modified natural polymer in the form of beads that used cellulose as the base material and that had carboxyl

groups as the functional group (the commercial name: CM Cellulofine C-200), each 5 ml, was suspended in purified water at room temperature and washed. Then, it was mixed with each 20 ml of a M/100 phosphate buffer (pH 4.5) and was subject to shaking at room temperature for 30 min. To each was added 250 mg of water-soluble carbodiimide hydrochloride, and it was stirred at room temperature for 30 min. Then, the sodium salt of ampicillin or cephalexin, 10 mg, was added and its immobilization treatment was conducted at room temperature for 5 h. After these beta lactam-immobilized cellulose beads of the carboxylic acid type were sufficiently washed with purified water according to the standard method, their antimicrobial activity against Staphylococcus aureus FDA209P was investigated according to the rapid evaluation method for antibiotic activities, when they displayed strong antimicrobial activity against Staphylococcus aureus FDA209P.

[0063]

Example 7 Grafted fibers with carboxyl groups Having Immobilized Ampicillin or Cephalexin by Use of 1,1-Carbonylbis-1H-(Carbodiimide)Imidazole (Water-Insoluble Carbodiimide)

10 pieces of the grafted fibers with carboxyl groups (1.0 x 1.0 cm), which were used in Example 1, were immersed in a M/10 phosphate buffer (pH 4.5), they were dehydrated and dried. These sections were placed in anhydrous dimethyl sulfoxide, 5 ml, and the sodium salt of ampicillin or cephalexin, 10 mg, was added, and it was immobilized

under anhydrous state at 5°C overnight. After the immobilization reaction, beta lactam-immobilized fiber sections of the carboxylic acid type were sufficiently washed with dimethyl sulfoxide. Similarly to Example 1, their antimicrobial activity against Staphylococcus aureus FDA209P was investigated according to the rapid evaluation method for antibiotic activities, which confirmed the manifestation of the antimicrobial activity.

[0064]

Example 8 Preparations Having Immobilized Ampicillin by Use of the Substrates Already Activated with Carbodiimide

Two types of commercially available substrate products that had previously been activated with carbodiimide (REACTIGEL (6X) and REACTI-GEL (HW-65F); Products from Pierce Inc.) were used to carry out the immobilization of ampicillin.

After fully wished, their antimicrobial activity against Staphylococcus aureus FDA209P was investigated similarly to Example 1 and confirmed the manifestation of the antimicrobial activity. Incidentally, the site of linkage was not confirmed.

[0065]

[Effects of Invention]

A polymer substrate product carrying thereon a low-molecular weight compound having a selective biological activity which is obtained according to the method of the present invention is directly contact with a target to exhibit the biological activity, without releasing or

diffusing such a low-molecular weight compound into the natural environment. Thus, the product of the present invention can perform a suitable therapeutic method to human, animals and plants without causing contamination of the environment and undesirable side effects.

As the polymer substrate, a grafted polymer may be used where the low-molecular weight compound is linked to a grafted chain which acts as a spacer. In such embodiment, the large number of the compound can be linked to both the terminal site and/or not terminal sites to provide a product having a high activity. In addition, the area to which the linked active compounds act can be enlarged.

[0066]

The product of the present invention maintains the selective, biological activity of the linked low-molecular weight compound which is immobilized. Thus, the activity of the product is stable and is not diffused and therefore will not affect on the matter other than target material. Microbes lived in the natural environment or normal cells in a living body will not unsuitably damaged and thus, this makes possible to conduct therapeutic method safely for a long time. According to the present invention, the product of the present invention can be used to a new treatment method for the natural environment, new cultivation method, and urgent medical therapeutic method.

[Brief Description of the Drawings]

[FIG. 1]

FIG. 1(A) shows the state where the amino group of

ampicillin was used to be immobilized to the carboxyl group of a polymer while the carboxyl group at the 2-position of ampicillin was maintained as it is.

FIG. 1(B) shows the state where the amino group of a polymer substrate was used to be immobilized to the carboxyl group at the 2-position ampicillin.

[FIG. 2]

FIG. 2 shows the result of evaluation of the antimicrobial activity of ampicillin-immobilized grafted fibers with carboxyl groups against Staphylococcus aureus FDA209P.

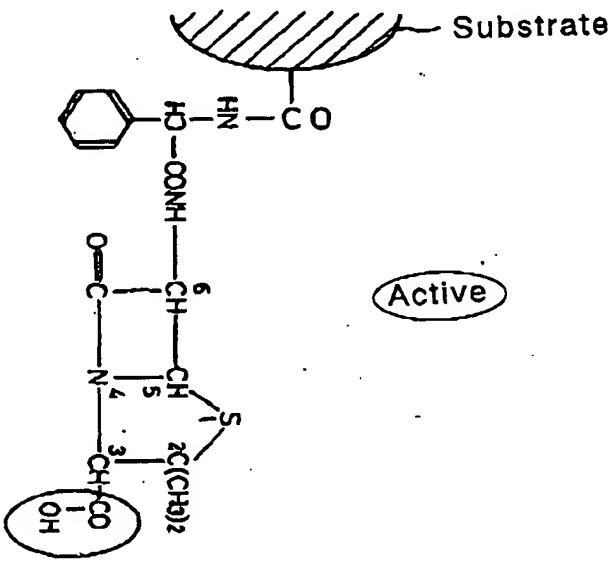
[FIG. 3]

FIG. 3 shows the result obtained when the antimicrobial activity of ampicillin-immobilized grafted fibers with carboxyl groups against Staphylococcus aureus FDA209P was evaluated in terms of the relationship between the area of sections of ampicillin-immobilized grafted fibers with carboxyl groups and their antimicrobial activity.

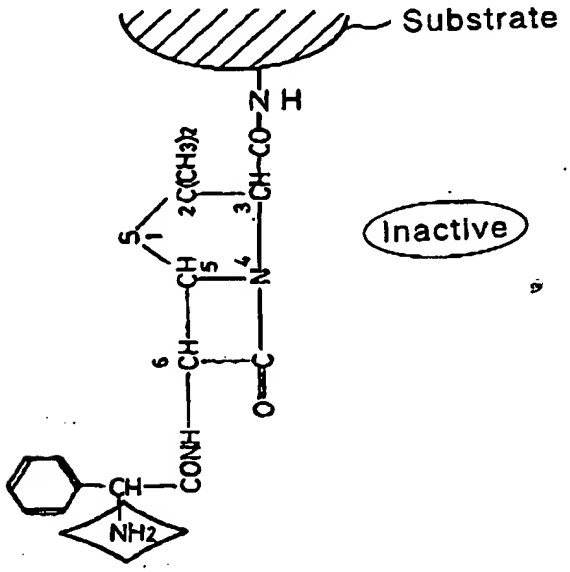
[Name of Document] Drawings

[Fig. 1]

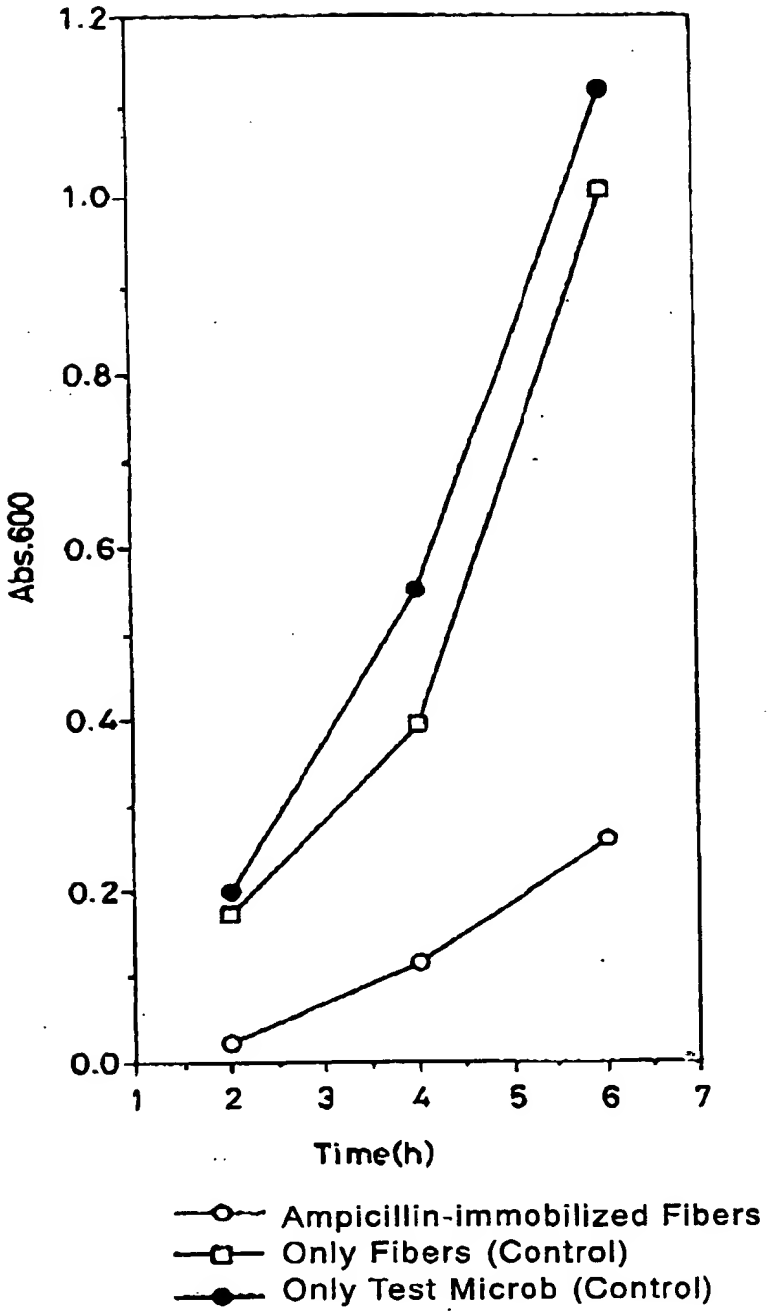
(A)



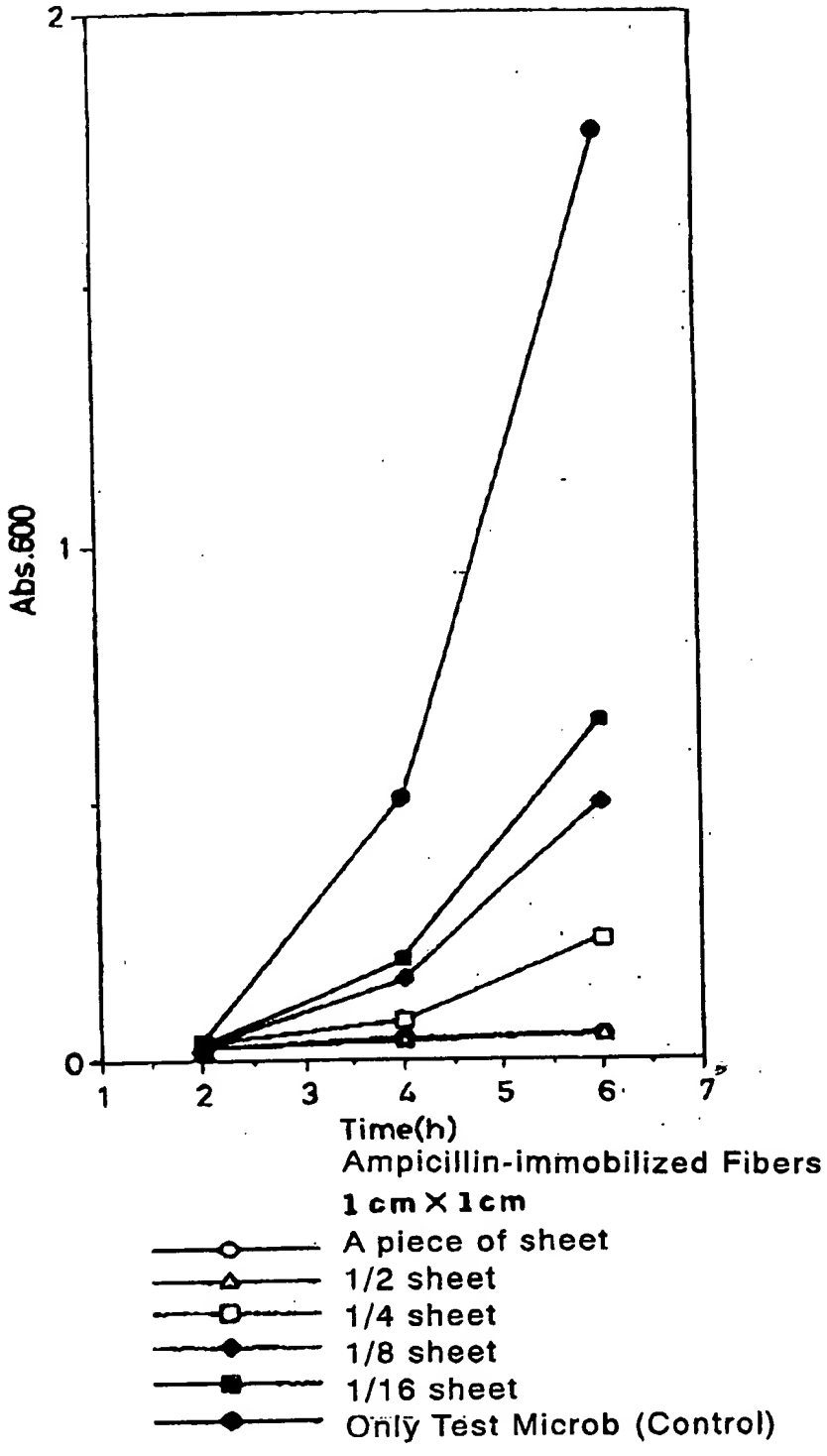
(B)



[Fig. 2]


1.2
1.0
0.8
0.6
0.4
0.2
0.0
Abs.600
1
2
3
4
5
6
7
Time(h)
Ampicillin-immobilized Fibers
Only Fibers (Control)
Only Test Microb (Control)

[Fig. 3]


2
1
0
Abs.600
1
2
3
4
5
6
7
Time(h)
Ampicillin-immobilized Fibers
1 cm × 1 cm
A piece of sheet
1/2 sheet
1/4 sheet
1/8 sheet
1/16 sheet
Only Test Microb (Control)

[Name of Document] Abstract

[Abstract]

[Problems] In order to prevent from causing undesirable side effects or contamination of the natural environment by the free diffusion of a low-molecular weight compound having pharmacological activities, the compound is linked to a polymer substrate while maintaining the activity of the compound to provide a polymer product which does not release the compound and exert the activity in the polymeric form.

[Means for Solution] A biologically active polymer product having a biologically active compound moiety immobilized to a polymer substrate through a covalent bond. The biologically active compound moiety such as an antibiotic exerts selective biological activity while being covalently bonded to the polymer substrate. A biologically active polymer product in which two or more biologically active compound moietys are linked to a polymer substrate through a graft chain or a pendant chain.

[Selected Drawing] None